



Human Chorionic Gonadotropin-Beta (hCG) MICRO-ELISA Test Kit

Prod. No.: T108
Pkg. Size: 96 Tests

Description

The MICRO-ELISA TOTAL BETA-hCG test is a solid phase sandwich-type enzyme immunoassay (ELISA) Diagnostic Kit for the *in vitro* quantitative determination of human chorionic gonadotropin (β hCG) concentration in human serum. The test can be used as an aid in the early detection of pregnancy.

Summary and Explanation of the Test

Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced during pregnancy by placental trophoblastic cells. The primary function of hCG is to maintain the corpus luteum. hCG is detected in the serum of pregnant women six to ten days after conception and its concentration doubles every 2 days for the first month and rises to peak levels of approximately 100,000 to 200,000 mIU/ml by the end of the first trimester. The hCG concentration then gradually decreases to 3,000 to 20,000 mIU/ml where it remains for the remainder of the pregnancy. After normal delivery the hCG concentration usually falls to undetectable levels within 1 month.

hCG consists of an alpha polypeptide subunit and a beta polypeptide subunit. Alpha subunits for the four human glycoprotein hormones (LH, FSH, TSH and hCG) are nearly identical. The beta subunit, which contains certain amino acid sequences common among these hormones, is distinct enough to produce the biological and immunological specificity of each hormone. The MICRO-ELISA TOTAL BETA-hCG test utilizes purified polyclonal and monoclonal antibodies to the beta subunit of hCG. The use of these antibodies allows the measurement of the total concentration of beta-hCG (both intact and free beta) and provides a system with high affinity to hCG with virtually no cross reactivity with LH, FSH or TSH.

Principle of the Procedure

The MICRO-ELISA TOTAL β hCG test is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). Antibody (rabbit polyclonal) to beta-hCG is coated to a plastic well (solid phase). Antibody (mouse monoclonal) to beta-hCG is contained in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test serum sample is allowed to react simultaneously with the coated and conjugated antibodies, resulting in the hCG molecule being sandwiched between the solid phase and enzyme-linked antibodies. After a 30 minute incubation at room temperature, the sample well is washed to remove unbound enzyme labeled antibody. An enzyme substrate-chromogen (hydrogen peroxide, H₂O₂, and tetramethylbenzidine, TMB) is added to the well and incubated for 15 minutes at room temperature, resulting in the development of a blue color. The addition of 1.0 N H₂SO₄ stops the reaction and converts the color to yellow. The intensity of the yellow color is directly proportional to the concentration of hCG in

the sample.

Reagents

Components In Each 96-Test

MICRO-ELISA β hCG Diagnostic Kit

- 96 wells, hCG ANTIBODY COATED **WELLS**: Coated with anti-hCG (rabbit polyclonal); contained in a pack with silica gel desiccant.
- 1 bottle, 22 ml, hCG ENZYME ANTIBODY **CONJUGATE**: Anti-hCG (mouse monoclonal) labeled with horseradish peroxidase in buffered protein solution; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives; contains FD&C red # 40 as coloring agent.
- 1 bottle, 12 ml, **SUBSTRATE-CHROMOGEN** Buffered hydrogen peroxide and 3,3',5,5'- tetramethylbenzidine (TMB) solution.
- 1 bottle, 12 ml **STOP SOLUTION** 1 N H₂SO₄
- 1 bottle, 50 ml **SAMPLE DILUENT**: Buffered protein solution; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives.
- 1 vial, 1 ml, **0 Miu/MI hCG CALIBRATOR**: Bovine serum; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives.
- 3 vials, 1 ml, hCG **CALIBRATORS**: hCG in bovine serum; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives; 200, 400 and 1,000 mIU/ml.
- 1 bottle, 60 ml, **WASH BUFFER CONCENTRATE (20X)**: Buffered detergent solution, contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives. Dilute bottle to 1200 ml with deionized water.

Additional Materials Required

Disposable tip precision pipets - 0.025, 0.1 and 0.2 ml.
microtiter plate reader.
Distilled or deionized water.

Storage and Stability

Store all components at 2°-8°C when not in use. The following components may be stored at ambient temperature: **WELLS**, **SUBSTRATE-CHROMOGEN**, **WASH BUFFER** and **STOP SOLUTION**. Expiration date printed on the kit indicates limits of stability.

The hCG ANTIBODY COATED **WELLS** are supplied in a resealable bag containing a desiccant and must be stored with the bag sealed to protect from moisture. Wells can be stored at 2°-30°C.



Chemical or Physical Indications of Instability

Alterations in the physical appearance of reagents, or results consistently outside the acceptable limits for control sera, may be due to reagent contamination or deterioration.

Instruments

Performance of the MICRO-ELISA β hCG test requires use of a precision microtiter plate reader with a wavelength of 450 ± 20 nm.

Specimen Collection and Preparation

Serum samples are used in the MICRO-ELISA β hCG Diagnostic Kit procedure. No special preparation of the patient is necessary; fasting is not required. Repeated freezing and thawing of specimens should be avoided. No additives or preservatives are necessary.

STORAGE: Specimens may be stored in a tightly sealed tube at 2° - 8° C for three days. If the serum is not assayed within 3 days, store frozen (-20° C) in a tightly sealed tube for up to 3 weeks. Specimens should be allowed to come to room temperature and should be mixed thoroughly by gentle inversion before assaying.

Micro-Elisa β hCG Procedure

Reagent Preparation

Dilute bottle of WASH BUFFER CONCENTRATE (20X) solution to 1200 ml with deionized water. Diluted wash solution will be stable until the expiration date stamped on the kit.

Preliminary Comments And Precautions

1. Patient sample may contain pathogens: treat all samples as potentially infectious.
2. Reagents contain thimerosal; avoid contact with skin.
3. Avoid contact with SUBSTRATE-CHROMOGEN (tetramethyl-benzidine) solution. It is harmful if inhaled or absorbed through skin (may cause irritation).
4. CAUTION: Source material used to prepare Calibrators was derived from human material. The material was tested using FDA-approved methods and found non-reactive for Hepatitis B Surface Antigen (HBsAg) by ELISA and non-reactive for HIV by ELISA. No known test method can offer total assurance that infectious agents are absent. HANDLE THESE REAGENTS AS IF THEY ARE POTENTIALLY INFECTIOUS. Information on handling human serum is provided in the CDC/NIH manual "Bio-safety in Microbiological and Biomedical Laboratories" (1984).

Procedural Notes

1. All test kit components used in an assay must be of the same master lot number. Materials should not be used after the expiration date shown on the package label. Components and test specimens should be at room temperature (18° - 30° C) before testing begins.
2. All calibrators, controls, and samples should be tested in duplicate simultaneously. The test samples and controls must be well mixed before use.

3. A separate disposable tip should be used for each sample to avoid cross-contamination. All pipetting steps should be performed with the utmost care and accuracy. Avoid contaminating the reagent pipette tip with the serum sample.
4. The duration of the incubation times must be the same for all wells within a run.
5. Run size should be limited to the number of samples that can be added to antibody coated wells within 5 minutes.
6. Samples should be pipetted to the bottom of the antibody-coated wells.
7. If microtiter reader is not capable of reading absorbances greater than 2.0, the color should be read after a shorter incubation time with the SUBSTRATE/CHROMOGEN, i.e., 10 minutes.

HELPFUL TIP: For quantitative results, dilute patient samples 1:101 by adding 10 μ l of patient sample to 1.0 ml of SAMPLE DILUENT and mix thoroughly. Run both the undiluted and the diluted samples. If the value of the undiluted samples is $>1,000$ mIU/ml, use the result of the diluted sample and multiply the result by 101. Dilution of the samples will allow you to calculate results up to 101,000 mIU/ml without having to repeat the assay.

Test Procedure

1. Place sufficient COATED WELLS in a holder to run 0.0 mIU/ml, 200 mIU/ml, 400 mIU/ml and 1,000 mIU/ml hCG CALIBRATORS, Quality Control Sera and patient samples in duplicate. Limit run size to the number of samples that can be pipetted in 5 minutes.
2. Pipet 25 μ l of the CALIBRATORS, Controls or Patient Sample to the corresponding COATED WELL.
3. Pipet or dispense 200 μ l of the ENZYME ANTIBODY CONJUGATE solution to all the wells and mix gently.
4. Incubate at room temperature (18° - 30° C) for 30 minutes \pm 5 minutes.
5. Decant or aspirate and discard liquid contents of all wells. SLAP the inverted wells on a clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.
6. Fill each well with diluted WASH BUFFER. Fill the wells to overflowing, you cannot cause any carryover between the wells. Decant or aspirate liquid contents of all wells. SLAP the inverted wells on a fresh clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.

WARNING: WASHING THE WELLS IS OF CRITICAL IMPORTANCE. Fill the wells to overflowing, you CANNOT cause any carryover between wells. You CANNOT over wash the wells. Completely decant or aspirate all of the liquid out of the wells. SLAP the inverted wells on a FRESH clean piece of absorbent paper AFTER EACH WASH. YOU CANNOT SLAP TOO HARD, REMOVE ALL OF THE LIQUID FROM THE WELLS.

7. Repeat step 6 three more times (for a total of 4 washes).
8. Fill each well with deionized water. Fill the wells to overflowing. Decant or aspirate liquid contents of all wells. SLAP the inverted wells on a fresh clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.



9. Pipet or dispense 100 µl (0.1 ml) of SUBSTRATE-CHROMOGEN solution into each well.
10. Mix thoroughly and incubate 15 minutes at room temperature (18°-30°C).
11. Pipet or dispense 100 µl (0.1 ml) of 1 N H₂SO₄ into each well and mix thoroughly.
12. Read the absorbance of each well at 450 ± 20 nm against water.

Calculation of Results

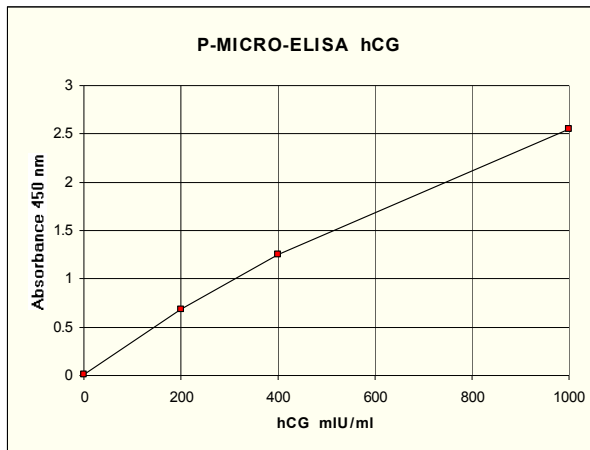
1. Calculate the mean value for each duplicate sample absorbance. Values for duplicate absorbances should be within 10% (or 0.02 absorbance units for absorbances less than 0.2).
2. Construct the standard curve by plotting the mean absorbance obtained for each hCG CALIBRATOR on the vertical (Y) axis versus the corresponding hCG concentration on the horizontal (X) axis, using rectilinear graph paper.
3. Connect the points with straight-line segments.
4. Using the mean absorbance for each sample, read the corresponding hCG concentration in mIU/ml from the curve. Multiply the value by the dilution factor if required.

EXAMPLE DATA
DO NOT USE IN PLACE OF CURVE
DETERMINED AT THE TIME OF ASSAY.

Specimen I.D.	A ₄₅₀	Mean A ₄₅₀	hCG(mIU/ml)
CALIBRATOR	0 mIU/ml	0.012, 0.014	0.013
CALIBRATOR	200 mIU/ml	0.698, 0.676	0.687
CALIBRATOR	400 mIU/ml	1.254, 1.240	1.247
CALIBRATOR	1,000 mIU/ml	2.580, 2.514	2.547

SAMPLES

# 1 (UNKNOWN #1)	0.042, 0.043	0.042	8.6
# 2 (UNKNOWN #2)	0.095, 0.101	0.098	25.2
# 3 (UNKNOWN #3)	0.705, 0.687	0.696	203.3



The range of this assay is 0 – 1,000 mIU/ml. For specimen with hCG concentrations beyond the standard curve (1,000 mIU/ml), repeat the test by diluting the specimen with the SAMPLE DILUENT. To obtain the final concentration, multiply the concentration of the diluted sample by the dilution factor.

Quality Control

Good laboratory practice requires that quality control specimen be run with each calibration curve to check the assay performance. Controls containing azide can not be used. Two controls with normal and elevated values should be used. Pooled human serum or commercially available control sera without azide are suitable. Any material used should be assayed repeatedly to establish mean values and acceptable ranges to assure proper performance.

Read the absorbance of the test solutions against a water blank. If the absorbance of the 0 mIU/ml CALIBRATOR exceeds 0.100 it is an indication of careless washing and the assay must be repeated.

Standardization

The MICRO-ELISA βhCG CALIBRATORS have been standardized against the World Health Organization International Standard Preparation (3rd IS 75/537).

Limitations of the Procedure

As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). These samples should not be assayed with the MICRO-ELISA βhCG test as erroneous results may be obtained. These conditions should be ruled out prior to clinical evaluation of test results.

The wash procedure (steps 6-8) is critical. Insufficient washing will result in poor precision and falsely elevated absorbances. The use of tap water for washing could result in a higher background absorbance.

FINAL REACTION STABILITY:

The spectrophotometric measurement should be made within 30 minutes after the addition of the H₂SO₄ solution.

Samples with elevated levels of hCG (up to 300,000 mIU/ml) will always assay as >1,000 mIU/ml when tested, and will not result in a "high dose hook effect". When it is necessary to measure levels of hCG greater than the 1,000 mIU/ml CALIBRATOR, the sample should be diluted with the SAMPLE DILUENT and re-assayed.

If pregnancy is suspected and an hCG value between 0 and 25 mIU/ml is obtained, repeat the test on a fresh sample in 48 hours to confirm pregnancy.



HCG has also been reported in conditions other than normal pregnancy including: threatened abortion, ectopic pregnancy, trophoblastic tumors, carcinomas of the stomach, liver, pancreas and breast, multiple myeloma and melanoma. These conditions should be ruled out prior to confirmation of pregnancy.

Expected Values

HCG is not normally detected in the serum of healthy men and healthy non-pregnant women. The concentration of hCG in the serum of pregnant women increases to 5 - 50 mIU/ml one week post implantation and continues increasing during the first ten weeks, reaching a maximum of 100,000 - 200,000 mIU/ml at the end of the first trimester. Values greater than 25 mIU/ml in serum should be considered positive. Positive results may be obtained as early as one week after implantation.

These values are consistent with those reported in the literature. It is recommended that each laboratory determine its own normal range.

Performance Characteristics of Test

Assay Specificity

Specificity of this test system was determined by studying the interference of luteinizing hormone (LH), follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH) in serum samples. The cross reactivity with LH was 1.7%, with FSH less than 0.01% and with TSH less than 0.01%

Assay Sensitivity

The sensitivity of this assay is defined as the smallest single value that can be distinguished from the zero calibrator. This value was calculated from the mean + two standard deviations for twenty-one replicates at the zero concentration. The calculated sensitivity is <5.0 mIU/ml.

Assay Reproducibility

Intra-assay reproducibility was determined by measurement of 21 replicates of three serum pools in a single run.

	Mean hCG (mIU/ml)	SD	%CV
Serum A	46.0	2.97	6.46
Serum B	124.3	6.03	4.85
Serum C	215.5	11.42	5.30

The interassay reproducibility was determined by duplicate measurement of three serum pools in thirty-two separate runs.

	Mean hCG (mIU/ml)	SD	% CV
Serum A	26.1	2.68	10.26
Serum B	99.9	6.75	6.76
Serum C	187.0	18.85	10.38

Assay Linearity

A study was performed diluting a serum sample containing an elevated level of hCG with the SAMPLE DILUENT to determine the linearity of the MICRO-ELISA βhCG.

Dilution Factor	hCG mIU/ml		
	Expected Value	Observed Value	% of Expected Value
Undiluted	-	>1000	-
1:101		5,422	
1:8	677.8	690.2	102 %
1:16	338.9	347.0	102 %
1:64	84.7	84.7	100 %
1:256	21.1	22.4	106 %
1:512	10.5	11.6	109 %

Assay Recovery

Two normal patient sera with known hCG values were spiked with 13.0, 26.0, 65.0 and 325.0 mIU/ml of hCG. The samples were assayed in duplicate.

Added hCG	hCG mIU/ml		
	Expected Value	Measured Value	% Recovery
0.0	125		
13	138	137	99 %
26	151	148	98 %
65	190	207	109 %
325	450	463	103 %
0.0	0		
13	13	14	108 %
26	26	28	108 %
65	65	65	100 %
325	325	355	109 %

Comparison to Other hCG Tests

Correlation studies on a random group of 60 serum samples with a range of values from <1 – 157,000 mIU/ml, were performed using the quantitative results from the MICRO-ELISA hCG Test and another ELISA hCG test. The correlation coefficient of the test results was 0.991.

		Slope	Y-Intercept	Correlation Coefficient
ELISA	n= 60	0.965	2.2	0.991

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